EXHIBIT 14

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Electronic particle counting for evaluating the quality of air in operating theatres: a potential basis for standards?

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Airborne particle counting in eight size ranges (0·5–>20 μm), by computerized electronic equipment, was compared with the numbers of bacteria-carrying particles (BCP) assessed by slit sampling in ultra-clean and turbulently ventilated operating theatres. In the ultra-clean theatre the number of particles of 5–7 μm size range correlated with BCP while peaks in the numbers of particles <3 μm and >15 μm corresponded with activity. Comparative relationships also occurred in the turbulently ventilated theatre but the use of this equipment in that environment cannot yet replace counts of airborne bacteria. We consider that electronic particle counting in the 0–20 μm size range may be used to judge the performance of a clean air operating theatre distribution system, including efficiency and integrity of the filter/seal systems and the presence or absence of entrainment of bacteria and other particles. The sampling techniques and analysis of particle concentration results described here may be a suitable basis for standards.

Clean air technology is increasingly used and provides a high quality environment in operating theatres. This follows the conclusions of the Medical Research Council (MRC) and Department of Health and Social Security (DHSS) multicentre trial which showed that ultra-clean air could reduce prosthesis-associated infection. Sepsis rates were found to be further reduced by the use of antibiotics in association with an ultra-clean air system (Lidwell *et al.* 1982). At the conclusion of this trial the DHSS set out to produce draft guidelines for the design and construction of ultra-clean air theatres that would be suitable for use in orthopaedic departments (Anon. 1986). As yet these guidelines have not been published and the draft recommendations define the performance criteria only in terms of a minimum uniform air-flow rate of 0·38 m/s beneath an area enclosed by a canopy.

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Whyte *et al.* (1983) suggested that bacteriological standards might define the performance specification of such systems. They considered that less than 20 bacteria-carrying particles per cubic metre of air (BCP/m^3) at a peripheral site and less than 10 BCP/m^3 at the operation site would be satisfactory irrespective of the detailed design of the installation. Others consider that 1 BCP/m^3 is a more acceptable level and also one that is achievable (Lidwell *et al.* 1982; Howorth 1984). It is not our purpose here to debate these contamination levels in relation to sepsis but rather to consider available test methods which may help in performance assessment. The development of an ultra-clean system that functions without the use of a partial wall or canopy (Seal 1988) has opened the way for using novel designs that meet proposed bacteriological performance criteria but not the design criteria of the draft DHSS recommendations.

If, in the future, comprehensive system performance is to be evaluated at the design stage

rather than at final installation, it will be necessary to define clearly appropriate test methods and procedures. Bacteriological evaluation of ultra-clean air environments by slit sampling, arguably the most effective absolute measure of contamination, is both time consuming, cumbersome and expensive. In addition, results are not available on the day of the test, thereby making assessment of the effects of adjustments and maintenance long drawn out.

In recent studies (Seal 1985; Clark *et al.* 1985) electronic particle counting in two size ranges (<0·5 μm and 0·5–5 μm) was investigated as a possible alternative to bacteriological assessment. The estimation of airborne particulate concentration levels by light scattering techniques is established as a method for evaluating air quality in different categories of 'clean rooms' in the electronic and pharmaceutical industries. This technology enables particle sizes and their concentrations to be measured directly and to be related to standards (Anon. 1988) for air quality relevant to these industries.

We have used electronic particle counting in experiments to assess air quality in operating theatres. Measurements of particle concentrations were made in eight size ranges (from 0·5–20 μm) during surgical operations in two operating rooms, one with and one without an ultra-clean air system. The results have been compared with bacteriological measurements made by slit sampling at the same time in each theatre.

Materials and Methods

OPERATING THEATRES

Particle concentration levels in eight size ranges (from 0·5–20 μm) and airborne bacterial counts were measured in two types of operating theatre. One theatre was equipped with a modern ultra-clean air system (Medical Air Technology Ltd.), that conformed to DHSS guidelines (Anon. 1986) and had a partial wall canopy (2·9 m × 2·9 m) on the ceiling to direct air unidirectionally toward the operating table at 0·4 m/s. The inflowing air was passed through HEPA filters and the airstreams beneath the canopy were designed to prevent entrainment of contamination from outside the clean zone. The air volume flow rate into the theatre was equivalent to 400 air changes per hour. The other theatre had an ordinary type of turbulent ventilation and had been used in a previous study (Clark *et al.* 1985); airflow was set at 20 changes per hour, entering the theatre peripherally at ceiling level and exhausting lower down. The airflows in this theatre provided a constant mixing of air within the room volume.

MEASUREMENT SITE

Particle and bacteriological samples were collected at the same time at a similar site 1·3 m to the side of the patient's shoulder and 1 m in front of the anaesthetist at a height of 0·8 m. In the ultra-clean air system this position was 'within the clean zone' at a peripheral site.

PARTICLE COLLECTION EQUIPMENT

A Kratel automatic air particle monitor (Semat (UK) Ltd., St. Albans, Herts., UK) with eight channels coupled to a sampling tube of polyvinyl chloride of fixed length of 12 m with an internal diameter of 0·8 cm, free of sharp bends and kinks, was used in this study to record particle concentrations between 0·5–20 μm. The air sampling rate was 28 l/min. Air samples were counted in each size range every minute before, during and after the operation and the results were immediately printed out.

Airborne bacteria were sampled with a Casella slit sampler operating at 700 l/min for 2 min in every 3 min period. The 7 in diameter nutrient agar plates from this sampler were

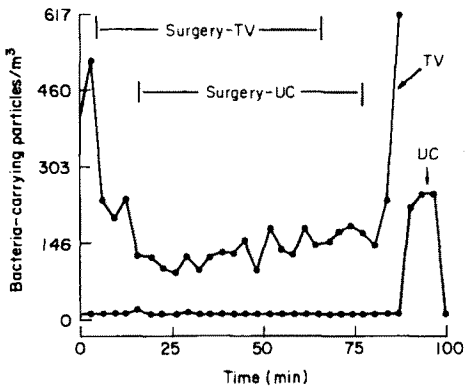

Fig. 1. Airborne bacterial levels (BCP/m³) before, during and after surgery in a turbulently ventilated (TV) and ultra-clean (UC) operating theatre.

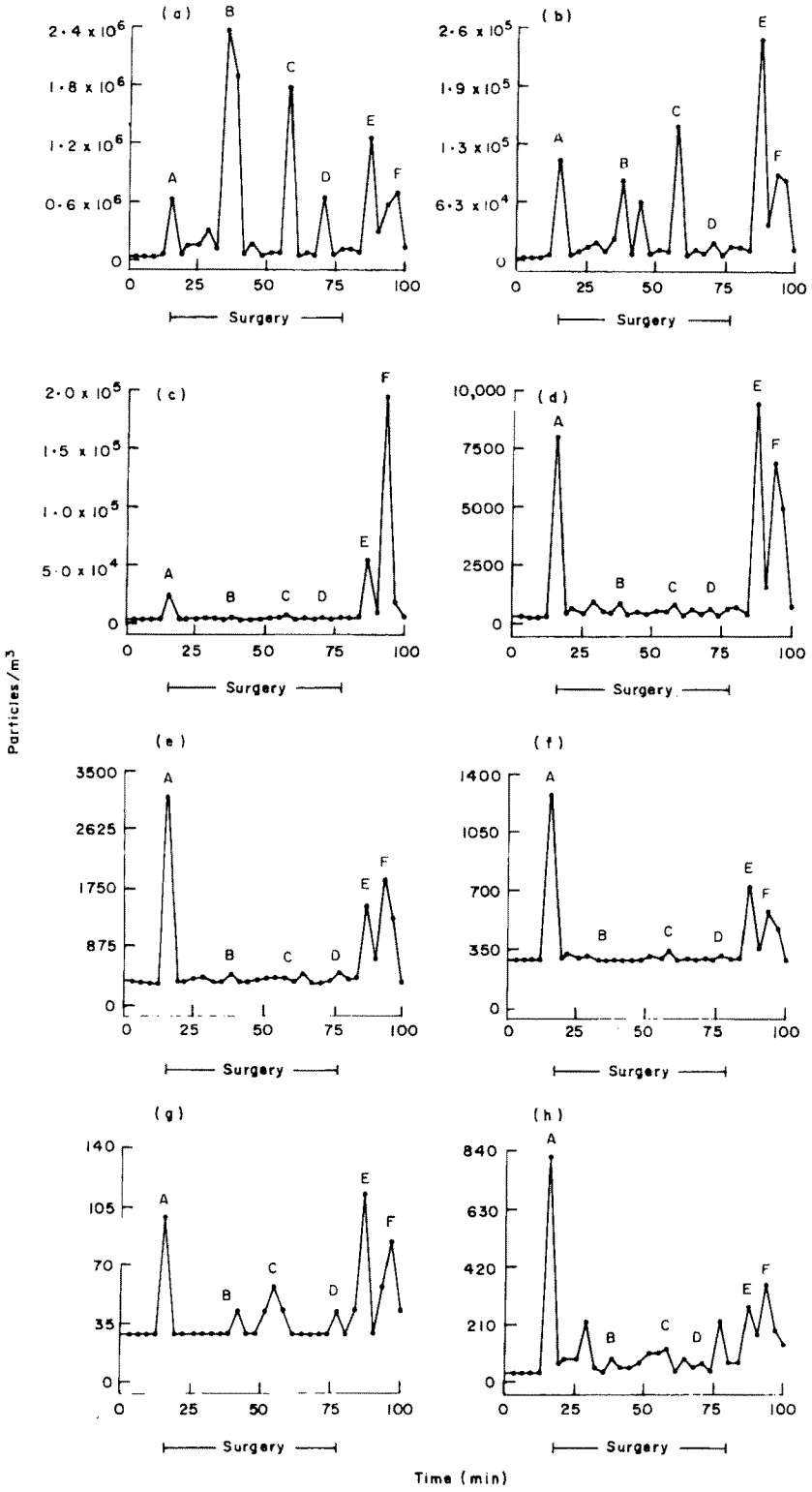

Fig. 2. Airborne particle concentration levels in eight size ranges for the ultra-clean theatre (UC) measured before, during and after surgery. Size ranges (μm): (a) 0·5–1·49; (b) 1·5–2·99; (c) 3·0–4·99; (d) 5·0–6·99; (e) 7·0–9·99; (f) 10·0–14·99; (g) 15·0–19·99; (h) >20. Activity performed: A, patient wheeled in; B, drilling bone; C, electric sawing of bone; D, cementing prosthesis in patient; E, anaesthetic activity, end of operation; F, patient leaves theatre.

incubated at 37°C for 24 h to give the total count for the 2 min period; this was then expressed as BCP/m³ of sampled air. The airborne particle concentrations were related directly to the relevant bacteriological measurement by expressing the particle count as an average over a similar 2 min period of each bacteriological measurement.

Results

Figure 1 contrasts the airborne bacterial levels before, during and after surgery in the ordinary turbulently ventilated and ultra-clean theatres. Figure 2 shows the particle concentration levels in eight size ranges for the ultra-clean theatre measured at the same time as the bacterial levels (BCP) illustrated in Fig. 1. This figure also shows the activity that took place and the relation of that activity to peaks in the numbers of airborne particles counted.

Figure 3 shows the size spectrum of particles in eight size ranges measured during surgery for the ultra-clean theatre contrasted with particle concentration specifications recommended for industrial clean rooms (Anon. 1988).

Table 1 gives results of mean particle counts at each size range for the ultra-clean and turbulently ventilated theatres.

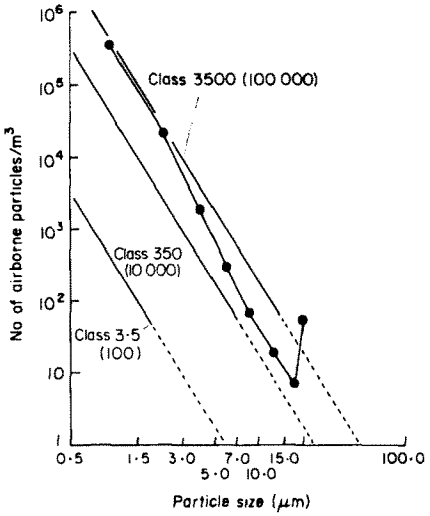

Fig. 3. Size spectrum of airborne particles measured in the ultra-clean theatre during surgery contrasted with the requirements for industrial clean room standards (Anon. 1988).

Table 1. Mean particle concentrations per m³ measured at a peripheral site before, during and after surgery in the ultra-clean and turbulently ventilated operating theatres

Size μm	Before Surgery	During Surgery	After Surgery
Ultra-clean			
0·5–1·4	3·1 × 10⁵	3·9 × 10⁵	5·6 × 10⁵
1·5–2·9	5·3 × 10⁴	2·2 × 10⁴	9·5 × 10⁴
3·0–4·9	2·6 × 10⁴	1·9 × 10³	5·6 × 10⁴
5·0–6·9	2·9 × 10³	2·8 × 10²	4·8 × 10³
7·0–9·9	7·1 × 10²	6·8 × 10	1·4 × 10²
10·0–14·9	2·3 × 10²	1·7 × 10	2·6 × 10²
15·0–19·9	2·8 × 10	6·0	6·9
>20	1·6 × 10²	5·1 × 10	2·0 × 10²
Turbulently ventilated			
0·5–1·4	5·3 × 10⁶	4·9 × 10⁶	5·3 × 10⁶
1·5–2·9	1·9 × 10⁵	3·9 × 10⁵	2·1 × 10⁵
3·0–4·9	4·6 × 10⁴	3·8 × 10³	5·6 × 10⁴
5·0–6·9	1·7 × 10⁴	1·2 × 10³	2·2 × 10⁴
7·0–9·9	2·2 × 10³	3·0 × 10²	2·6 × 10³
10·0–14·9	3·3 × 10²	9·5 × 10	3·3 × 10²
15·0–19·9	8·5 × 10	4·2 × 10	7·1 × 10
>20	1·1 × 10²	7·4 × 10	9·2 × 10

STATISTICAL ANALYSIS

Correlation coefficients were calculated between the average numbers of BCP at each time interval, of 2 min in every 3, and the particle counts at the same time in the size ranges 0·5–1·5, 1·5–3·0, 3·0–5·0, 5·0–7·0, 7·0–10·0, 10·0–15·0, 15–20, and >20 μm. These are shown in Fig. 4.

The relationship between particle size (5·0–7·0 μm) and BCP that gave the highest correlation

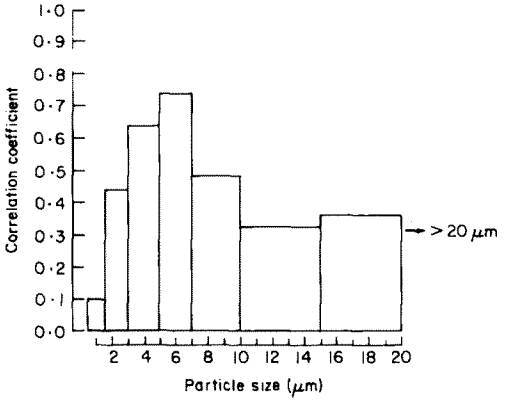

Fig. 4. Correlation coefficients between BCP recoveries and particle counts in the eight size ranges illustrated in Fig. 2.

coefficient (0·74) was explored for statistical significance by permutation analysis. All 33 counts of BCP measured were varied in time with respect to the equivalent particle counts for 10 000 permutations, and the correlation coefficient was calculated on a DEC 20 computer for each occasion. This determined whether the temporal positions of the BCP measured occurred by chance, or not, in comparison with the particle counts. This analysis showed that the likelihood of the BCP measured at the particular time intervals, with respect to their equivalent particle counts, being due to chance was less than 0·001, i.e. 1 in 1000.

Discussion

The airborne bacterial concentrations found in each theatre in this study were typical of those for turbulently ventilated and ultra-clean theatres (Williams *et al.* 1966; Whyte *et al.* 1983). The particle concentrations in the ultra-clean theatre in the eight size ranges shown in Fig. 2 indicate that peaks in concentration were consonant with the various activities of the theatre personnel and the size ranges 0·5–3·0 and 15–20 μm are particularly sensitive to these activities.

In the 3–15 μm size range the particles may be expected to include many skin scales which carry bacteria that are shed from the theatre team and patient (Noble *et al.* 1963). For the ultra-clean theatre the statistical analysis shows that there is a significant relationship between particles in the size range 5·0–7·0 μm and the BCP counted. This demonstrates that counts of particles in the size range 5·0–7·0 μm may well provide a valid alternative to BCP counts as an indication of airborne bacterial contamination in the vicinity of the operation site in the ultra-clean theatre.

It has been shown previously in an ultra-clean theatre that measurement of particles in the 0·5–5·0 μm range gave peaks that correlated with airborne bacteria during the disruption of the ultra-clean conditions with the arrival and removal of the patient (Seal 1985). In that study peaks of particles were also found during surgery that did not correlate with any peak in airborne bacteria. These peaks were thought at the time to be due to non-bacteria carrying particles. Our results confirm these previous observations. Particles less than 3 μm and greater than 15 μm clearly corresponded with activity even when bacteria were not detected.

In an ultra-clean theatre there is a 'core' of clean filtered air which passes rapidly over the surgical team and patient before being dispersed to the periphery and recycled through high efficiency filters; bacterial and particle entrainment should not occur from outside to within the 'clean' area if the system is working properly. In an ultra-clean system without entrainment, contaminants will encounter the particle sampling device only once. In a turbulently ventilated system mixing will allow the concentration of contaminants to reach higher and more uniform levels which may re-present to the sampler at a frequency dependent on the make-up air dilution rate. It is therefore expected that samplers in the ultra-clean system would be more sensitive to transient concentration levels produced by activity. Particle concentration levels measured at 0·5–3·0 μm and >15 μm in the turbulently ventilated theatre (Table 1) showed less relation to activity than those in the ultra-clean system (Fig. 2 and Table 1). The use of multiple point sampling, rather than single point sampling that we used, might give more accurate and consistent results. Because of the background effect of non-bacteria carrying particles we cannot recommend this alternative for bacteria counting in turbulently ventilated conditions.

Average spectral distributions of the airborne particles in the ultra-clean theatre during surgery are shown in Fig. 3 where they are compared with the requirements for industrial clean rooms (Anon. 1973). This shows that an effect can be gained equivalent to a Class 3500 (100 000) 'industrial clean room'. For monitoring air quality during surgery in ultra-clean air conditions, particularly with newly installed or experimental systems (and routinely thereafter at regular maintenance periods), a particle count of less than 1000/m^3 in the 5–15 μm range should indicate satisfactory air cleanliness and that entrainment of bacteria, or particle leakage through filters and seals, is not occurring. The advantage of this technique is that the results are immediately available and the effects of differing numbers of staff, changes in airflow velocity or temperature differential (Whyte & Bailey 1978) and different types of theatre configuration can be immediately assessed.

In all particulate monitoring systems the most obvious sampling error is particle loss in tubing. Considerations of all the forces and their magnitudes acting on particles in transit along sampling tubes are complex involving parameters such as impaction, tubing electrical conductivity, turbulence levels in the airflow, particle electrostatics and aerodynamic shape. Considerable information exists (Zweers 1983; Liu *et al.* 1985; Anon. 1985) to quantify losses in different types and sizes of tubing, up to at least 30 m in length, for a range of particle sizes. PVC tubing is frequently used and the particle drop-out performance is exceeded only by expensive anti-static tubing. With built-in aerosol sampling multiplexing systems these effects are accounted for in calibration and programming of the equipment. In the present investigation it is estimated that the greatest error due to drop-out of large particles would have been up to 20% and for those up to 3 μm diameter 12%. The estimated losses of particles greater than 15 μm has poor statistical validity due to the relatively low numbers of such particles. Such losses are likely to remain constant and will not substantially affect the size spectrum of particles in relation to clean room standards. In our investigations these losses did not affect 'event' discrimination by the particle counting systems. The advent of new 'low loss' tubing (i.e. Bev-A-Line) can be expected to increase measurement accuracy.

Current trends in clean air technology are based on 'multi-point' sampling sites feeding a single particle counter with microprocessor analysis of contamination levels for large clean areas. Such systems are being built into clean room facilities to enable continuous on-line monitoring of environmental air quality. They are immediately able to detect and warn of changes in contamination levels that may result from technical breakdowns or 'off limit' procedures. In some installations the ultra-clean air systems are automatically controlled and adjusted in relation to pre-set airborne particle levels. There would seem to be a clear application for this technology to evaluate ultra-clean air systems in operating theatres, in relation to possible performance standards.

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